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January 5, 1938

Dr. F. R. Sabin
The Rockefeller Institute for Medical Research
New York, N. Y.

Dear Doctor Sabin:

I wish to acknowledge your letters of December 17th and 28th. The package which you sent about the middle of December arrived safely in due time. Some of the material which you returned will be quite useful to me.

I was very sorry to learn from your letter of December 28th that you have discovered tubercle bacilli in the phosphatides from A-10 and A-14. The original solutions of these phosphatides were all treated in exactly the same manner, that is to say the ethereal solutions were filtered under carbon dioxide pressure through new Chamberland filters. The resulting filtrates were sparklingly clear. Of course this may not be any real criterion that the filtrates did not contain some bacilli, but I should imagine the amount must have been very small.

I still have some A-14 phosphatide on hand and will refilter its ethereal solution as you suggest and send you a sample of the preparation after this treatment.

It is now many years since I stained bacteria and I cannot, therefore, give any worthwhile suggestions about this procedure, but judging by the properties of the phosphatide, I would suggest the following method of fixing on the slide. Some of the phosphatide should be dissolved in ether or chloroform and the solution should be spread on the slides and the solvent allowed to evaporate. The resulting film should be fixed by heat in the usual manner and then stained. The phosphatide is so easily emulsified in water that it is rather surprising that any of it remains on the slide after staining with hot acueous fuchsin. The phosphatide is so insoluble in alcohol that I would expect very little effect on the film by the acidified alcohol treatment. However, if most of the material is removed during this operation, it must be due to the effect of the acid present in the alcohol. It would seem, however, if any clumps of bacteria are present that some of them ought to be fixed on the slide so that they could be observed after the staining process. As soon as the new phosphatide preparation has been prepared, I shall be glad to send it to you.

Very sincerely.

R. J. Anderson